

dride), continuous currents of very considerable power may be obtained when the internal resistance is diminished sufficiently by using cells of considerable magnitude; *e.g.*, when made of the stoneware and inner porous vessels usually employed for Grove's cells, the porous vessel being cemented into the outer stoneware vessel (by paraffin wax or other unattacked material) in such a fashion as to divide it into three compartments separated one from the other by porous dividing walls; the acid and alkaline fluids being placed in the two outermost compartments, and the innermost one being filled with a solution of a neutral salt, *e.g.*, sodium sulphate. A large variety of analogous cells of more or less power can thus be formed by using different organic and inorganic reducing substances soluble in alkali, *e.g.*, ferrocyanides, hydrosulphites, opianates, &c.

“On Kreatinins. I. On the Kreatinin of Urine as distinguished from that obtained from Flesh Kreatin. II. On the Kreatinins derived from the Dehydration of Urinary Kreatin.” By GEORGE STILLINGFLEET JOHNSON, M.R.C.S., F.C.S., F.I.C. Received May 5,—Read June 16, 1887.

PART I.

I was induced to undertake this investigation by a careful observation of the action of picric acid and potassium hydrate upon normal human urine at the boiling temperature.

The introduction of picric acid as a test for sugar in urine is due to my father, Dr. George Johnson, who accidentally discovered the production of a very dark colour on the addition of picric acid to a portion of saccharine urine which had been previously boiled with potassium hydrate. He made this observation in November, 1882. The dark colour was found to be due to *reduction* of potassium picrate to potassium picramate by glucose at the boiling temperature, and in presence of potassium hydrate. The reaction had been described by C. D. Braun nearly twenty years previously (“*Ueber die Umwandlung der Pikrinsäure in Pikraminsäure und über die Nachweisung des Traubenzuckers*,” ‘*Fresenius, Zeitschrift*,’ 1865), but it had not hitherto been applied to any practical purpose.

Having observed the extreme delicacy of the test, and the ease with which the reaction is effected, my father determined to introduce it to the notice of the medical profession, not merely as a trustworthy qualitative test of extreme delicacy, but also as a means of estimating quantitatively the actual amount of glucose in diabetic urines. In this latter object I did my best to assist him, and our united efforts resulted in the elaboration of a new, easy, and accurate method for

determining sugar in urine, which is fully described in Dr. G. Johnson's volume of 'Medical Lectures and Essays,' and also in the last edition of Dr. Roberts' work on 'Urinary and Renal Diseases.'

Reaction of the Picric Acid and Potash Test in Normal Human Urine.

As soon as the picric acid and potash test was introduced to my notice, I applied it to normal human urine, and found that it gave indication of the presence therein, if not of glucose, at all events of a reducing substance capable of reducing yellow potassium picrate to red potassium picramate in presence of potassium hydrate at the boiling temperature.

After the elaboration of the test as a quantitative one, many specimens of urine from men in perfect health were examined by its means, and we never met with a single specimen which gave no reducing action, though, as would be expected, the reduction was more marked in concentrated specimens, and the smallest quantity of reducing substance was found in the urine of patients suffering from diabetes insipidus, in which cases the specific gravity of the secretion was very low.

In most urines from healthy individuals, of the average specific gravity (1.020), the amount of reduction exerted upon picric acid in presence of potassium hydrate at the boiling temperature corresponded with that which would have been produced by a solution of glucose containing 0.6 *grain per fluid ounce*; whilst the quantity of reducing agent indicated by the method of Fehling or Pavy is always slightly greater, the cupric oxide reduction of normal human urine expressed in terms of glucose averaging 0.75 *grain per fluid ounce*.

But is this normal reducing agent glucose? and if not, what is it? The object of the present research is to answer these questions.

Observations which negative the Hypothesis that the Reducing Agent of Normal Urine is Glucose.

Dr. Robert Kirk ('Lancet,' June 16, 1883) was, I believe, the first to publish the fact that healthy urine gives some indication of reducing picric acid in presence of potash *at the ordinary temperature*, whereas glucose effects no reduction until the temperature approaches the boiling point. This is an important distinction.

Again, after repeated experiments, I have never once succeeded in producing alcohol and carbon dioxide from normal human urine by the action of yeast, even when the solution was artificially concentrated before introducing the yeast, and the liquid containing the ferment was kept under the most favourable conditions of temperature, &c.

Repeated Observations have confirmed the Existence of Reducing Agents in Normal Human Urine.

It was probably owing to the impossibility of accounting otherwise for the reducing action which normal urine invariably exerts upon cupric oxide in boiling alkaline solutions, that physiological chemists were led to assert that dextrose is present in the urine of healthy men.

In a paper published in the Medico-Chirurgical Society's 'Transactions' (vol. 63, p. 222), Dr. F. W. Pavy, F.R.S., writes as follows:—"The reducing action before the addition of acetate of lead is due partly to uric acid and partly to the small amount of sugar naturally present in urine. It is doubtful if there is any other body worthy of consideration to exert any sensible reducing effect." And Dr. Pavy finds that one-fourth of the total reduction of copper oxide by normal urine is due to uric acid. This result Dr. Pavy arrived at by estimating the cupric oxide reduction of the urine before and after precipitation by lead acetate, which removes from solution the uric acid, but not the other reducing agent to which three-fourths of the total reducing effect must be ascribed, and which Dr. Pavy concludes is sugar. Brücke's views on this subject are too well known to need comment.

Amongst the known substances other than glucose, which reduce potassium picrate to picramate in boiling alkaline solutions, are—

(1.) Potassium ferrocyanide, a salt which is not likely to be found in urine, since it is devoid of medicinal properties, and is therefore not likely to be administered.

(2.) Sulphides of the alkali metals. I have shown ('Chemical News,' vol. 47, 1883, p. 87) that in boiling *dilute* solutions of potassium hydrate, albumen yields potassium tetrathionate, and not potassium sulphide, and as potassium tetrathionate does not reduce potassium picrate in boiling dilute solutions of potassium hydrate, and as a very dilute solution of potassium hydrate is employed in the quantitative estimation of glucose by picric acid, viz., 30 minims of the liquor potassæ of the British Pharmacopœia, diluted to 4 drachms, there is no possibility of reduction of picric acid by alkaline sulphides, formed by the action of potassium hydrate upon unoxidised sulphur compounds in the urine at the boiling temperature.

Kreatinin has been suggested by Dr. Oliver as the reducing agent of normal urine ('Bedside Urinary Testing'). The sample of kreatinin which he examined was sold by Messrs. Hopkin and Williams. I have examined a portion of this sample, and found that it was very deficient in reducing power. Allowing most liberally for its presence in the urine, only about one-twentieth of the total reduction of cupric oxide effected by that secretion in its normal condition could be accounted for by this substance.

The reducing action of kreatinin upon cupric oxide in boiling solutions containing caustic alkali has been long known to German chemists. Thus, Kühne draws attention to this fact at page 505 of his 'Lehrbuch der Physiologischen Chemie.'

As regards the *amount* of cupric oxide reduction usually attributed to kreatinin by German physiological chemists, I may quote a recent paper by Professor E. Salkowski, in the 'Centralblatt für die Medicinischen Wissenschaften,' March, 1886, in which the reduction due to uric acid and kreatinin combined is estimated as varying from one-fifth to one-sixth of the total cupric oxide reduction effected by normal urine, the remainder being attributed to other substances, and probably to compounds of glycuronic acid (Glykuronsäureverbindungen).

The Reducing Agent of Normal Urine is disintegrated by prolonged Boiling with Dilute Solution of Potassium Hydrate.

Whilst examining the reactions of the reducing agent in normal urine, I found that by prolonged boiling with dilute potassium hydrate solution, about three-fourths of the copper oxide reducing power of the urine is lost, the remaining one-fourth being due to survival of the uric acid. The urine which had been subjected to this treatment did not reduce picric acid at all, for uric acid has no reducing action upon picric acid in boiling alkaline solutions.

The reducing agent of normal urine is therefore disintegrated by prolonged ebullition with potassium hydrate. On comparing the behaviour of solutions of glucose in the same circumstances, I have since found that such solutions lose their reducing action upon cupric oxide with far greater rapidity than the reducing agent of normal urine.

In the 'British Medical Journal,' (March 17th, 1883), will be found a table of results of some determinations of the reducing action of normal urines upon picric acid and cupric oxide respectively, in which the effect of prolonged boiling with diluted potassium hydrate solution upon the reducing agent of normal urine is visible

I.	II.	III.	IV.
Total indication by picric acid.	Total indication by ammonio- cupric method.	Indication by ammonio-cupric method, after boiling with potash.	Difference between II and III, normal reducing agent.
gr. per 1 fluid oz.	gr. per 1 fluid oz.	gr. per 1 fluid oz.	gr. per 1 fluid oz.
(1.) 0·6	0·909	0·276	0·63
(2.) 0·5	0·607	0·09	0·517
(3.) 0·35	0·546	0·145	0·401
(4.) 0·8	1·245	0·437	0·808

at a glance. In this table all the reductions are expressed in terms of glucose.

Summing up all the evidence, there is no doubt that a reducing agent is present as a normal constituent of the urine of healthy men, and that it confers upon normal urine the property of reducing cupric oxide to the same extent as if it held in solution (on the average) 6 grains of glucose in every 10 fluid ounces of urine, or 1.34 grams *per litre*. But, considering the impossibility of causing alcoholic fermentation to take place in solutions of this reducing agent, and its property of reducing picric acid to some extent in presence of potassium hydrate at the ordinary temperature, its identity with glucose appears to be very doubtful. Further doubt is thrown upon this identity by the fact that if a solution of mercuric chloride be added to normal urine and afterwards potassium hydrate, the yellowish precipitate which forms, becomes grey in a few minutes by reduction at the ordinary temperature, whereas glucose does not effect reduction of mercuric oxide in presence of potassium hydrate without application of heat in less than one hour.

Endeavours to Isolate the Reducing Agent of Normal Urine.

Having examined qualitatively the reactions of the reducing agent of normal urine, and having found reasons to doubt its identity with diabetic sugar, I next proceeded to employ various precipitants with a view to its removal from the complex fluid in which it is dissolved.

Normal lead acetate, basic lead acetate, baryta-water, solution of ammonia, alcohol, were all employed in turn, but the filtrates in each case reduced picric acid as strongly as before precipitation, showing that the precipitants employed had failed to remove the reducing agent from solution.

Finally I succeeded in removing the whole of the normal reducing agent from urine by complete precipitation with strong aqueous solution of mercuric chloride.

Previous Researches on the Action of Mercuric Chloride upon Normal Human Urine.

It has long been known that an aqueous solution of mercuric chloride produces in *all* specimens of normal urine a flocculent precipitate, when added in sufficient excess. But the state of our knowledge as to the nature of the substance or substances thus precipitated is very unsatisfactory.

Dr. John Greene, of Birmingham, published in the 'British Medical Journal' (May 10th, 1879) a research in which he describes the isolation from this precipitate, produced by mercuric chloride in normal urine, of a white flocculent albumen-like substance, precipitable by lead acetate as well as by mercuric chloride, and refusing to

dialyse through animal membranes. Dr. Greene analysed this substance, with the following results:—

Carbon	34.52
Hydrogen	5.71
Nitrogen	12.58
Oxygen	47.19
	100.00

The empirical formula indicated by these numbers is $C_{16}H_{30}N_5O_{16}$, which requires—

Carbon	35.03
Hydrogen	5.47
Nitrogen	12.77
Oxygen	46.73
	100.00

The main object of Dr. Greene's research was to prove that the precipitate produced by mercuric chloride in normal urine contains a large proportion of organic nitrogen, and that corrections might be made, in the determination of the urea in urine by the hypobromite method, by deducting so much nitrogen for every gram of mercury precipitate yielded by the original urine.

As, however, Dr. Greene rejected everything in the precipitate by mercuric chloride in normal urine which is not also precipitated by lead acetate, he of course missed the normal reducing agent altogether, and must have also considerably under-estimated the total nitrogen contained in the mercury precipitate, for the reducing agent of normal urine is highly nitrogenous, as will presently appear.

Maly ('Ann. Chem. Pharm.,' vol. 159, p. 279) describes a method for obtaining kreatinin hydrochloride from the urine of man or the horse by precipitation with mercuric chloride. His method consists in concentrating the urine to one-third of its original bulk, then precipitating by lead acetate and filtering, whereby the uric acid and Greene's substance are removed. The filtrate is freed from lead by sodium carbonate or sulphuretted hydrogen, neutralised after a second filtration by acetic acid or sodium carbonate, and then precipitated by mercuric chloride. The precipitate is washed and then decomposed by hydrogen sulphide under water, and the filtrate is decolourised by animal charcoal and evaporated. The residue, on being re-crystallised once or twice from alcohol, yields pure kreatinin hydrochloride in hard shining prisms (*vide* Watts's 'Dictionary,' Suppl. 2, p. 393).

In spite of these researches, mercuric chloride has recently been again recommended as a precipitant for *albumen* in urine, and the

precipitate produced by this reagent in normal urine has been attributed to *urea*. Enough, however, has been said to prove that the precipitate produced by mercuric chloride in normal urine is of a very complex nature.

Reaction of Normal Human Urine with Strong Solution of Mercuric Chloride.—The Author's Researches.

In all my experiments with a view to separate the reducing agent from normal urine, I have employed a solution of mercuric chloride in water, saturated at the temperature of the laboratory (16° C.).

In my first experiments I filtered the urine immediately after adding to it one-fourth of its volume of the cold saturated mercuric chloride solution, and always found that the filtrates still contained the reducing substance in solution.

I soon observed, however, that after separating the flocculent amorphous precipitate first produced by mercuric chloride, the filtrate did not long remain clear, but after about half an hour a second precipitate began to form, having a granular appearance, and continuing to separate out for many days. On filtering from time to time, and examining the reducing action of the filtrate, it was found that the reducing power progressively diminished as more and more of the mercury salt separated out from the solution. In short it soon became evident that the reducing agent of normal urine may be completely removed from that liquid by precipitation with mercuric chloride.

Although the complete precipitation of the reducing substance is very slow if mercuric chloride be added alone, if we add to fresh unconcentrated urine one-twentieth of its volume of a cold saturated solution of sodium acetate, then one-fourth of its volume of cold saturated solution of mercuric chloride, and filter immediately, the filtrate deposits the whole of the normal reducing agent as mercury salt in about forty-eight hours. The precipitation is known to be complete when the filtrate from the second mercury precipitate remains clear, even on the addition of more solution of sodium acetate and mercuric chloride. It will then be found that the permanently clear filtrate is without reducing action upon both potassium picrate and cupric oxide in boiling alkaline solutions.

The first precipitate produced by mercuric chloride in normal urine is amorphous and flocculent, and has some resemblance to coagulated albumen. Finding that it contained no reducing agent, I have deferred the study of this precipitate for the present, but a cursory examination of it showed that it contained uric acid, probably as mercuric urate, besides the substance described by Dr. Greene, which is precipitated by lead acetate. When decomposed by hydrogen sulphide under water, the filtrate from mercuric sulphide is acid in reaction,

contains much chlorine, but no phosphoric acid, and yields a gummy mass on evaporation. It exerts no reducing action either upon cupric oxide or potassium picrate in boiling alkaline solutions.

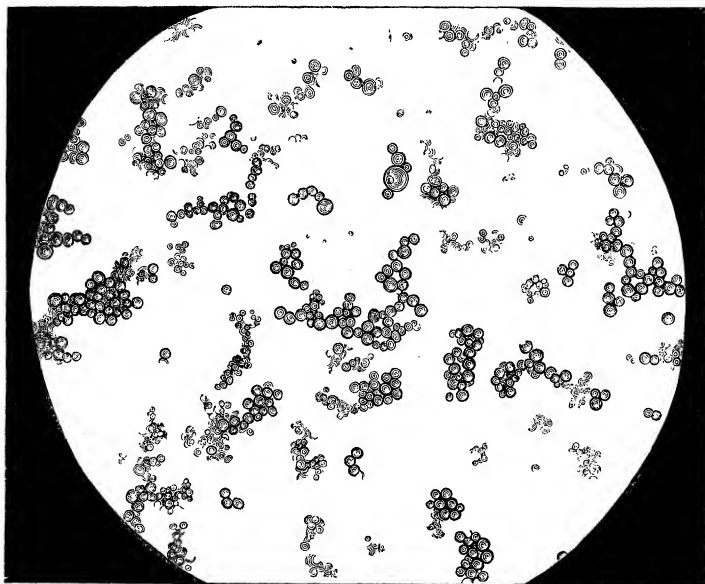
Satisfied, therefore, that the granular precipitate which gradually separated out from the filtrate from the above amorphous substance contains the whole of the normal reducing agent of urine, I confined myself to the examination of that compound.

Physical Properties of the Mercury Salt of the Reducing Base of Urine.

I have described this precipitate as granular, and it certainly appears upon superficial observation to be a crystalline substance, as indeed might be expected from its gradual formation, but microscopic examination reveals some curious facts in connexion with it.

Examined under a quarter-inch object glass, the substance is at once seen to be perfectly homogeneous, and often has the appearance of minute crystals united together in stellate groups; but under a one-sixteenth inch object glass these stellate groups are seen to be composed of a number of very minute and perfectly spherical masses. I have watched the growth of these spherules from a minute point to a little globe resembling an oil-globule. The

FIG. 1.



Microphotograph of Spherical Mercury Salt of Kreatinin, precipitated from fresh normal human urine. $\times 1500$ diameters.

mode of formation of this compound appeared to me so remarkable that I have prevailed upon my friend Mr. Herbert Jackson to execute some microphotographs of the spherical mercury salt of the reducing base of urine.

This spherical mercury salt is very sparingly soluble in cold water. When recently precipitated it dissolves with great ease in hydrochloric acid, but is insoluble or nearly so in acetic acid.

Chemical Reactions and Decompositions of the Spherical Mercury Salt.

Aqueous solution of ammonia does not blacken the spherical compound, but if the salt be thrown into boiling water, the sediment which remains undissolved is immediately blackened by ammonia-water, so that a portion of the mercury in this compound is reduced to the mercurous condition by contact with water at 100° C. Therefore, the spherical mercury salt must be washed with cold water, and must be dried *in vacuo* over sulphuric acid, in order to avoid this decomposition.

Moistened with solution of potassium hydrate at the ordinary temperature, the compound gradually becomes black by reduction, a compound ammonia escaping at the same time in abundance.

Suspended in cold water and treated with hydrogen sulphide, the compound first becomes yellow and finally black (mercuric mercury), and if the stream of gas be continued, nothing remains undissolved but mercuric sulphide. The solution is acid in reaction, and reduces both potassium picrate and cupric oxide in presence of potassium hydrate at the boiling temperature (hydrochloride of the reducing base).

Having convinced myself that the spherical compound is a definite substance, I proceeded to ascertain the weight of it which is yielded by a known volume of urine, and to subject the salt to analysis with a view to ascertain its composition.

Whilst determining the quantity of the spherical compound obtained by precipitation from known volumes of urine, I was of course accumulating a store of material from which to prepare large quantities of the reducing base itself for subsequent examination. The method which I adopted was as follows :—

A quantity of fresh human urine is examined for albumen and sugar, and, if found normal, its volume is exactly noted. The reaction to litmus is then ascertained, and also the amount of reduction of picric acid (or cupric oxide) which it is capable of effecting. Next the specific gravity of the specimen is observed, after which I add to it one-twentieth of its volume of a cold saturated solution of sodium acetate, and from one-third to one-fourth of its volume of cold saturated mercuric chloride solution. There is no need to concentrate the urine by evaporation. The mixture is now immediately

filtered to separate the flocculent amorphous mercury precipitate, and the filtrate is received into a large glass vessel capable of holding about 60 litres. It is better not to operate with more than a litre of urine at a time, because if a larger volume be taken there is risk of formation of spherical mercury salt before the filtration is complete, and of its retention upon the filter. But the mercuric chloride acts so effectually in preventing putrefaction that sample after sample may be treated as above directed, and filtered into the same receptacle, in which the spherical compound accumulates and awaits the convenience of the operator. When a sufficient quantity has accumulated, it is thrown upon a filter, washed with cold distilled water till the washings are only slightly troubled by silver nitrate, and the washed compound is then dried over sulphuric acid *in vacuo* and weighed.

The following tables will give an idea of the scale upon which I have operated, and will also show how the weight of mercury salt obtained is proportional to the amount of reduction exerted by the specimens examined:—

Table I.

No. of men contributing.	Volume of urine.	Specific gravity at 16° C.	Picric acid reduction expressed as glucose.	Reaction to litmus.
4	c.c. 5,600	1·021	gr. per fluid oz. 0·6	Feebly acid
3	1,600	1·023	0·65	Neutral
3	900	1·023	0·87	Feebly acid
4	1,200	1·018	0·75	Feebly acid
2	1,100	1·023	0·75	Acid
2	1,000	1·027	0·88	Feebly acid
2	790	1·025	0·87	Acid
3	1,100	1·023	0·87	Acid
3	750	1·031	1·00	Acid
1	900	1·031	1·00	Acid
1	1,000	1·026	0·85	Acid
1	2,000	1·026	0·85	Acid
1	1,300	1·027	0·90	Acid
Many.....	15,400	1·017	0·62	Acid

Table II.

Volume of urine in c.c.	Specific gravity at 16° C.	Picric acid reduced (as glucose).	Reaction to litmus.	Vol. of sat. HgCl ₂ added.	Vol. of sat. NaAg added.
c.c. 1,690	1·017	gr. p. fluid oz. 0·7	Acid	c.c. 430	c.c. 60
1,350	1·025	0·85	Acid	400	50
2,180	1·022	0·75	Acid	600	100
1,755	1·025	1·2	F. acid	450	60
1,150	1·022	0·9	Acid	320	60
1,560	1·025	0·9	Acid	400	50
1,730	1·026	0·85	Acid	450	50
1,430	1·022	1·0	Acid	375	45
1,910	1·020	0·9	Acid	500	70
1,420	1·016	0·7	F. acid	410	50
1,000	1·020	0·85	Acid	300	40
1,380	1·020	0·8	Acid	350	50
1,000	1·025	1·1	Acid	300	40
1,715	1·025	1·0	Acid	450	70
1,760	1·025	1·0	Acid	500	80
1,410	1·018	0·75	Acid	400	60
1,785	1·024	0·7	Acid	500	70
1,000	1·022	0·8	Acid	300	50
1,140	1·019	1·0	Acid	310	50
1,535	1·022	1·0	F. acid	500	70
1,495	1·022	0·85	Acid	500	70
1,500	1·026	0·75	Acid	500	70
1,410	1·025	1·0	Acid	500	70
1,950	1·020	0·7	Acid	800	100
770	1·025	1·0	Acid	300	40
2,000	1·019	0·9	Acid	800	100
1,600	1·020	0·8	Acid	700	100
40,625	..	..	..	12,345	1,725

The total volume operated on (Table I) was thus 34,640 c.c. The mean specific gravity was 1.020, and the mean reduction of picric acid (expressed in terms of glucose) was equivalent to 0.67 grain of glucose per fluid ounce.

The spherical mercury salt obtained from the above urine, after being washed with cold water and dried *in vacuo* over sulphuric acid, weighed 198 grams. This is at the rate of 5.7 grams of the spherical compound to each litre of urine.

The mean specific gravity at 16° C. of the above samples (Table II) of normal human urine is 1.022, and the mean reduction of picric acid is equivalent to that which would be effected by a solution containing 0.86 grain of glucose per fluid ounce.

The washed and dried spherical mercury salt from the above 40,625 c.c. of normal urine weighed 293.13 grams, which is equivalent to 7.19 grams per 1 litre of urine.

It is apparent from the above tables that the weight of mercury salt obtained from an equal volume of urine is proportional to the amount of reduction effected by the secretion, and that both have a tendency to increase with the specific gravity of the latter.

The following observations made upon my own urine during the past winter, illustrate some interesting points in connexion with the precipitation of normal urine by mercuric chloride:—

My weight at the time of the experiment was 70.08 kilos. I was in good health, in active work both mental and bodily, and consuming an ordinary mixed diet. All the samples had the normal acid reaction to litmus, and were free from albumen and sugar.

The total urine of each twenty-four hours, having been measured, was mixed with a cold saturated solution of sodic acetate, and then precipitated with one-fourth of its volume of cold saturated solution of mercuric chloride. Both first and second precipitates were collected on filters previously counterpoised, washed with cold water, dried *in vacuo* over H_2SO_4 , and weighed.

The total volume of urine during the six days amounted to 8930 c.c.

Average per 24 hours.....	1488 c.c.
Maximum in ,, ...	1790 ,,
Minimum in ,, ...	1230 ,,

	Volume of urine.	Weight of 1st Hg. ppt.	Weight of 2nd Hg. ppt.
	c c.	grams.	grams.
December 29 to 30	1790	5·355	9·076
„ 30 to 31	1360	9·238	9·930
„ 31 to January 1	1575	7·300	8·758
January 1 to 2	1230	6·715	8·176
„ 2 to 3	1700	4·985	8·932
„ 4 to 5	1275	7·735	7·955
Six days	8930	41·328	52·827

Hence it appears that the mercury salt of the reducing base is far more constant in quantity than the amorphous mercury-compounds first precipitated. Also in my case the average weight of *kreatinin* passed in twenty-four hours amounted to 1·77 grams, or 25/1000000 of the body-weight.

Analysis of the Spherical Mercury Salt from Urine.

As precipitated from urine, the spherical mercury salt has a fawn colour, due to enclosure of urinary colouring-matter. It was therefore deemed advisable to re-precipitate the compound in order to obtain it pure for analysis. With this object in view, the thoroughly washed compound is suspended in water and subjected to a stream of hydrogen sulphide until completely decomposed. The deep yellow filtrate from the mercuric sulphide is digested for some days with purified animal charcoal (which must be quite free from lime salts), and is then mixed with solutions of sodium acetate and mercuric chloride. A very pure compound is obtained, if the decolourised solution is largely diluted before adding the mercuric chloride, in which case any precipitate which forms at once may be separated by filtration, after which the filtrate gradually deposits the pure spherical compound in a colourless condition. Having been washed with cold water and dried *in vacuo* over sulphuric acid, the pure mercury salt was analysed with the following results:—

By Combustion with Copper Oxide.

(a.) 1·1920 gram of the Hg salt gave 0·1500 gram of H_2O .
and 0·3755 „ CO_2 .

Equivalent to 0·01666 gram hydrogen.
and 0·10241 „ carbon.

Hence Hg salt contains { hydrogen, 1·39 per cent.
 { carbon, 8·592 „

- (b.) 1.3302 grams of Hg salt gave 0.1522 gram H_2O .
and 0.4187 „ CO_2 .

Equivalent to 0.016811 gram of hydrogen.
and 0.114191 „ carbon.

Hence Hg salt contains $\begin{cases} \text{hydrogen, 1.26 per cent.} \\ \text{carbon, 8.584 „} \end{cases}$

The *nitrogen* was estimated in this compound and in all the analyses recorded in this research by a modification of Dumas' method. The substance to be analysed is introduced in a boat behind the copper oxide, instead of being mixed with the latter. By this means the copper oxide can be ignited strongly in a stream of carbon dioxide, before adjusting the measuring tube for the nitrogen. Residual carbon left in the boat at the end of the combustion is burnt off by oxygen generated by heating potassium chlorate in a second boat placed behind that which contains the substance to be analysed.

The following results were obtained:—

- (a.) 0.7728 gram of Hg salt gave 44.83 c.c. of nitrogen, measured at 0°C . and 760 mm. P., equivalent to 0.0560385 gram of N.

Hence the Hg salt contains 7.25 per cent. nitrogen.

- (b.) 0.6228 gram Hg salt gave 36.29 c.c. of nitrogen, measured at 0°C . and 760 mm., equivalent to 0.0453625 gram of N.

Hence the Hg salt contains 7.28 per cent. of N.

The *mercury* was determined in two ways: 1st, by dissolving the Hg salt in HCl and precipitating the solution by H_2S ; and 2ndly, by suspending the Hg salt in water and decomposing by H_2S . The close concordance between the results proves how very completely the mercury salt may be decomposed by hydrogen sulphide under water.

- (a.) 1.0882 gram of Hg salt dissolved in boiling HCl gave by precipitation with H_2S 0.7868 gram of HgS, equivalent to 0.67827 gram of metallic mercury.

Hence the salt contains 62.33 per cent. of Hg.

- (b.) 0.8232 gram of Hg salt, suspended in water and decomposed by H_2S , gave 0.5996 gram HgS, equivalent to 0.5169 gram of mercury.

Hence the salt contains 62.79 per cent. of Hg.

The *chlorine* was determined (1) by decomposing the Hg salt under water with H_2S and precipitating the filtrate with AgNO_3 ; (2) by igniting the Hg salt with pure sodium carbonate, dissolving in water, acidulating with HNO_3 , and precipitating with AgNO_3 . The following results were obtained:—

- (a.) 0.8070 gram of the Hg salt gave 0.5226 gram of silver chloride, equivalent to 0.1285 gram of chlorine, or 15.92 per cent. of Cl.

(b.) 0·8232 gram of Hg salt gave 0·5356 gram of AgCl, equivalent to 0·1325 gram of chlorine.

The salt therefore contains 16·09 per cent. of chlorine.

The empirical formula derived from the results of these analyses is $C_{16}H_{28}N_{12}O_6Hg_7Cl_{10}$.

The simplest rational formula is $4(C_4H_5HgN_3O \cdot HCl) \cdot 3HgCl_2 \cdot 2H_2O$.

	Theory.		Found.	
Carbon.....	8·575		8·588	$\left\{ \begin{array}{l} 8·592 \\ 8·584 \end{array} \right.$
Hydrogen...	1·251		1·325	$\left\{ \begin{array}{l} 1·39 \\ 1·26 \end{array} \right.$
Nitrogen....	7·503		7·275	$\left\{ \begin{array}{l} 7·26 \\ 7·28 \end{array} \right.$
Oxygen.....	4·288		4·247	(By difference).
Mercury	62·528		62·560	$\left\{ \begin{array}{l} 62·79 \\ 62·33 \end{array} \right.$
Chlorine	15·855		16·005	$\left\{ \begin{array}{l} 16·09 \\ 15·92 \end{array} \right.$
	100·000		100·000	

The apparent molecular weight of the mercury salt (confirmed subsequently) is 2239, and it contains 20·19 per cent. of the base $C_4H_7N_3O$.

The crude spherical mercury salt, as obtained directly from urine, yielded on combustion—

Carbon, 8·46 per cent.

Hydrogen, 1·17 „

Preparation and Properties of the Hydrochloride of the Reducing Base of Urine.

In order to prepare the hydrochloride of the reducing base of urine, the crude spherical mercury salt is suspended in water and completely decomposed by hydrogen sulphide. The deep yellow acid filtrate from the resulting mercuric sulphide is then decolourised as completely as possible by very pure animal charcoal, and the nearly colourless filtrate from the charcoal is left to evaporate spontaneously *in vacuo* over sulphuric acid. Large well-formed prisms with pyramidal apices separate out, which only need washing with a little strong alcohol to separate a small quantity of dark tarry matter, which usually adheres to the bottom of the vessel. They need no re-crystallisation.

This ready crystallisation of the hydrochloride of the reducing base is doubtless due to the great purity of the mercury salt which is attained by separating the amorphous compounds first precipitated from the urine by mercuric chloride by immediate filtration.

I have worked Maly's process (*vide supra*) exactly as he directs,

and found that, notwithstanding the preliminary precipitation of the concentrated urine with lead acetate, a tarry mass is obtained after the H_2S treatment of the mercury precipitate, which stands much in need of the re-crystallisation from alcohol recommended by Maly, before it will yield distinct crystals. If, however, the precipitate first formed by mercuric chloride in Maly's process be separated by immediate filtration, and only the precipitate produced in the filtrate on standing be decomposed by H_2S , all goes smoothly and well. The preliminary precipitation by lead acetate and the artificial concentration of the urine are quite useless.

The crystals of the hydrochloride of the reducing base of urine, obtained as above, are quite permanent in the air, and their weight is unaltered by exposure to a temperature of 100°C .

0.3708 gram of the crystals (not re-crystallised) gave 0.3512 gram of silver chloride, equivalent to 0.086881 gram of chlorine, or 23.43 per cent. Cl.

The formula $\text{C}_4\text{H}_7\text{N}_3\text{O} \cdot \text{HCl}$ requires 23.74 per cent. Cl.

The crystals are excessively soluble in water, and also to a great extent in alcohol.

Mixed in aqueous solution with mercuric chloride, there is no precipitate, but on adding solution of sodium acetate, the spherical mercury salt separates out.

Mixed in aqueous solution with mercuric chloride and potassium hydrate, there is first a whitish precipitate, which dissolves in excess of the potassium hydrate, but the solution becomes turbid again after a few seconds with formation of a yellowish precipitate, which immediately afterwards becomes black by spontaneous reduction.

Mixed in aqueous solution with Nessler reagent, a bright yellow precipitate separates out, which rapidly becomes black by spontaneous reduction, a compound ammonia being evolved at the same time.

When an alcoholic solution of the hydrochloride of the reducing base of urine is mixed with excess of an alcoholic solution of platinic chloride, a yellow crystalline platinum salt is immediately precipitated. These crystals appear dendritic under the microscope. They are anhydrous, and permanent in the air.

When dissolved in water, and spontaneously evaporated, a fine orange-coloured prismatic platinum salt is formed, which loses water at 100°C ., becoming opaque and of a lemon-yellow tint. The same orange-coloured prisms are obtained by spontaneous evaporation of a mixture of the hydrochloride of the reducing base with platinic chloride in aqueous solution.

The determination of the *water of crystallisation* in these orange-coloured prisms shows that they contain (on the average) 5.32 per cent. of H_2O .

Thus 0.4960 gram of Pt salt, crystallised from aqueous solution and air-dried, lost 0.0260 gram at 100° C., equivalent to 5.24 per cent.

5.1396 grams of Pt salt, prepared as above, lost 0.2706 gram at 100° C., corresponding with 5.27 per cent.

0.8332 gram of Pt salt, re-crystallised from aqueous solution and air-dried, lost 0.0454 gram at 100° C., corresponding with 5.44 per cent.

The *anhydrous platinum salt of the reducing base* was submitted to analysis with the following results:—

0.4866 gram of platinum salt gave 0.1188 gram of water, equivalent to 0.0132 gram of hydrogen, or 2.71 per cent. of H, and

0.2680 gram of CO_2 , equivalent to 0.0736 gram of carbon, or 15.12 per cent. carbon, and left

0.1514 gram of platinum, corresponding with 31.11 per cent. of Pt.

0.4172 gram of Pt salt gave 0.1060 gram of water, equivalent to 0.01177 gram of hydrogen, or 2.82 per cent., and

0.2336 gram of CO_2 , equivalent to 0.06371 gram of carbon, or 15.27 per cent. of C.

The *chlorine* was determined by mixing the platinum salt with pure caustic lime, igniting, dissolving in diluted nitric acid, filtering from the spongy platinum (which is weighed), and precipitating the chlorine in the filtrate as AgCl .

0.5535 gram of Pt salt gave 0.7460 gram of AgCl , equivalent to 0.1845 gram of chlorine, corresponding with 33.333 per cent. Cl, and left

0.1715 gram of platinum, corresponding with 30.984 per cent. of Pt.

0.5430 gram of Pt salt gave 58 c.c. of nitrogen, measured at 0° C. and 760 mm., equivalent to 0.072 gram of nitrogen, or 13.25 per cent.

0.3060 gram of platinum salt gave 32.85 c.c. of nitrogen, measured at 0° C. and 760 mm., equivalent to 0.04106 gram of nitrogen, or 13.41 per cent.

These results agree well with the formula $2\{\text{C}_4\text{H}_7\text{N}_3\text{O.HCl}\}.\text{PtCl}_4$.

	Required.			Found.		
Carbon	15.047			15.190	{	15.12
						15.27
Hydrogen . . .	2.507			2.760	{	2.71
						2.82
Nitrogen	13.166			13.330	{	13.25
						13.41

	Required.		Found.	
Oxygen	5.017		4.340	(By difference)
Platinum . . .	30.878		31.047	{ 30.984 31.11
Chlorine	33.385		33.333	
	100.000		100.000	

The above being the formula of the anhydrous platinum salt of the reducing base of urine, it appears that the formula of the orange-coloured prisms obtained by spontaneous evaporation of the aqueous solution, is $2(\text{C}_4\text{H}_7\text{N}_3\text{O}.\text{HCl}).\text{PtCl}_4.2\text{H}_2\text{O}$. This formula requires 5.34 per cent. of water of crystallisation, whilst the mean of the three determinations given above indicates the presence of 5.32 per cent.

Preparation and Properties of the Reducing Base—Kreatinin of Urine.

The reducing base or kreatinin of urine is obtained in the free state by treating the concentrated aqueous solution of its hydrochloride (prepared as above from the spherical mercury salt) with excess of very pure hydrated lead oxide at the ordinary temperature.

The crystallised hydrochloride should be dissolved in about fifteen times its weight of cold water, and an excess of perfectly pure recently precipitated lead hydrate added to the solution, which should be kept stirred constantly for about twenty minutes at the ordinary temperature. After the liquid has acquired a strongly alkaline reaction, it will be found to filter clear through paper. The filtrate is perfectly free from chlorine and colouring matter, and yields the urinary kreatinin on spontaneous evaporation *in vacuo* over sulphuric acid in the crystalline condition. It is better not to dilute the filtrate by adding the washings from the lead oxychloride to it, but these may be evaporated separately by heat.*

The same sample of the spherical mercury salt of the urinary kreatinin may be made to yield three different substances according to the treatment adopted in the separation of the kreatinin from it. The following details of an experiment conducted upon the large scale will best exemplify this statement.

260 grams of the spherical mercury salt of the urinary kreatinin

* The lead hydrate employed for liberating the reducing kreatinin of urine from its hydrochloride must be free from *basic lead nitrate*. When this compound is present, the alkaline filtrate, though free from chlorine, is found to hold in solution both lead and the radicle of nitric acid; moreover, the lead cannot be removed from solution by animal charcoal, even at the boiling temperature, but must be separated by hydrogen sulphide, after which the filtrate from the lead sulphide exhibits an acid reaction, and deposits crystals of kreatinin nitrate on evaporation.

I have found it best to employ lead hydrate precipitated from the *acetate* by ammonia.

was decomposed by hydrogen sulphide in presence of water, special care being taken to avoid too great a rise of temperature by constant agitation in a closed bottle during the action of the gas upon the precipitate.

After the action was complete the solution was set aside for three days in a tall glass cylinder, to allow the mercuric sulphide to settle. As much as possible of the clear and supernatant liquor was then decanted off from the precipitate, and set to evaporate *in vacuo* over sulphuric acid. Hard anhydrous crystals of kreatinin hydrochloride resulted. After being washed with a little cold alcohol, these crystals were dissolved in about fifteen times their weight of cold water, the solution was well stirred with pure lead hydrate, and filtered. The filtrate when evaporated *in vacuo* over sulphuric acid, deposited needle-shaped crystals of a base for which I propose the name of *efflorescent kreatinin*, whose properties and composition will be described shortly. This efflorescent kreatinin, being obtained from the spherical mercury salt of urine as far as possible without any application of heat, I believe to be the true natural kreatinin of urine.

The washings from the lead oxychloride, &c., after the above treatment were evaporated at 60° C. on a copper plate kept hot by steam. A number of *anhydrous* square tables were obtained on cooling; which when dissolved in cold water yielded an alkaline solution which deposited efflorescent kreatinin on evaporation *in vacuo* over sulphuric acid.

The third substance is obtained as follows:—

The washings from the mercuric sulphide in the above experiment were concentrated over steam. The concentrated liquor was diluted, decolourised by animal charcoal, and filtered. The filtrate was again concentrated over steam, and finally evaporated *in vacuo* over sulphuric acid. The resulting crystals of kreatinin hydrochloride did not differ in outward appearance from those obtained from the solution to which no heat had been applied; but, when decomposed by pure lead hydrate after solution in fifteen parts of cold water—in short, under conditions as nearly as possible identical with those present in the case of the hydrochloride crystallised from cold aqueous solution—instead of efflorescent kreatinin, anhydrous square or oblong tabular crystals are obtained, similar in form to the tabular crystals resulting from the evaporation of an aqueous solution of the efflorescent kreatinin of urine at 60° C., but more transparent than the latter, and differing from them in that their cold aqueous solution deposits anhydrous tabular crystals again on spontaneous evaporation, instead of efflorescent crystals.

This tabular anhydrous kreatinin, which re-crystallises unaltered from its cold aqueous solution on evaporation *in vacuo* over sulphuric

acid, I shall speak of in this paper as *tabular kreatinin α of urine*, whilst the tabular crystals which yield efflorescent kreatinin under similar treatment will be alluded to as *tabular kreatinin β of urine*, the term *efflorescent kreatinin* being reserved for the natural base.

It will be apparent from the following details of experiments that these three varieties of urinary kreatinin are convertible into one another at the will of the operator.

Suppose we start with *efflorescent kreatinin*. This substance crystallises in splendid transparent square prisms, often upwards of an inch in length, which, however, begin to lose their transparency when freed from extraneous moisture within half an hour in summer weather, and very rapidly even in winter upon mere exposure to common air. After complete efflorescence the crystals retain their original form without any tendency to crumble, and then resemble porcelain in appearance.

Now if these effloresced crystals are re-dissolved in the smallest possible quantity of *cold water*, the solution deposits efflorescent kreatinin again on evaporation *in vacuo* over sulphuric acid.

But if the effloresced kreatinin be dissolved in water at 60° C., and the evaporation be continued at that temperature till the crystallising point is reached, the crystals deposited on cooling are *tabular kreatinin β of urine*, anhydrous crystals, which yield efflorescent kreatinin on spontaneous evaporation of their cold aqueous solution over sulphuric acid. And if the effloresced kreatinin be dissolved in water at 100° C., the solution, evaporated *in vacuo* over sulphuric acid (in case the water present is in sufficient quantity to hold all the kreatinin in solution at the ordinary temperature), deposits crystals of *tabular kreatinin α of urine*, which re-crystallises unchanged from cold aqueous solution.

Again, if tabular kreatinin α of urine be dissolved in a larger volume of water at 60° C. than is necessary to hold it in solution at the ordinary temperature, and if the solution be kept at 60° C. for one hour, then allowed to cool and evaporated *in vacuo* over sulphuric acid, efflorescent kreatinin crystallises out.

Analysis of Urinary Kreatinin.

The determination of the water of crystallisation in the efflorescent kreatinin is not easy, on account of the rapidity of the efflorescence when the crystals are quite free from extraneous moisture.

The results of analysis, however, prove that the composition of the crystals is represented by the formula $C_4H_7N_3O \cdot 2H_2O$.

The mean of the two determinations gives 23.87 per cent. H_2O , whilst the formula $C_4H_7N_3O \cdot 2H_2O$ requires 24.17 per cent.

Weight of efflorescent kreatinin taken.	Loss of weight at 100° C.	Loss per cent.	Required for $C_4H_7N_3O \cdot 2H_2O$.
gram. 0·3195 0·4230	0·0770 0·1000	24·10 23·64	24·17 24·17

When a weighed quantity of the effloresced kreatinin of urine is dissolved in boiling water, and the solution evaporated to dryness *in vacuo* over sulphuric acid, the weight of tabular kreatinin α obtained is identical with that of the effloresced kreatinin taken.

This fact indicates that the percentage composition of the two bodies is identical; a view which is fully supported by the results of their ultimate analysis.

Description of substance.	Weight of substance in grams.	Weight of hydrogen found.	Weight of carbon found.	H in 100 parts.	C in 100 parts.
Tabular kreatinin α of urine.....	0·3170	0·02015	..	6·35	..
Tabular kreatinin α of urine.....	0·2080	..	0·088036	..	42·32
Effloresced kreatinin of urine....	0·3968	0·0260	0·16541	6·55	41·68

Description of substance.	Weight of substance in grams.	Vol. of nitrogen at 0° C. and 760 mm.	Weight of nitrogen in grams.	N in 100 parts.
Tabular kreatinin α of urine	0·1754	c.c. 52·64	0·066032	37·64
Effloresced kreatinin of urine	0·1540	45·73	0·05736	37·24

The following table shows that these results agree well with the formula $C_4H_7N_3O$:—

	Found in tabular K.		Found in effloresced K.		Required for $C_4H_7N_3O$.
Carbon.....	42·32		41·68		42·478
Hydrogen.....	6·35		6·55		6·194
Nitrogen.....	37·64		37·24		37·168
Oxygen (difference)	13·69	 (difference)	14·53		14·160
	<u>100·00</u>		<u>100·00</u>		<u>100·00</u>

Supported by the analyses of mercury and platinum salts, and of the hydrochloride, these analyses leave no doubt that the empirical formula of the reducing base of urine is $C_4H_7N_3O$.

Gold Salt of the Kreatinin of Urine.

Both the tabular kreatinin α of urine and the efflorescent kreatinin of urine, when dissolved in dilute hydrochloric acid and mixed with a concentrated aqueous solution of auric chloride, yield large thin plates of kreatinin-auric chloride. This salt is remarkably stable. The crystals are permanent in the air, possess a brilliant golden-yellow lustre, and undergo no change at $100^\circ C.$, except a temporary darkening of colour, which disappears again on cooling.

The analysis of this salt confirms the view that the molecular weight of the reducing kreatinin of urine is 113.

Analysis of Gold Salt of the Efflorescent Kreatinin of Urine.

Weight of kreatinin-auric chloride taken in grams.	Weight of gold found.	Weight of chlorine found.	Weight of gold in 100 parts.	Weight of Cl in 100 parts.
0.2670	0.1165	0.08324	43.63	31.17

These results agree with the formula $C_4H_7N_3O.HCl.AuCl_3$.

	Found.	Theory.
Au	43.63	43.43
Cl	31.17	31.37

Determination of the Solubility in Water of the Efflorescent Kreatinin and of Tabular Kreatinin α of Urine.

Some solution of efflorescent kreatinin of urine, which was depositing crystals over sulphuric acid at $14^\circ C.$, was poured off from these crystals into a tared dish, weighed, and evaporated to dryness *in vacuo* over sulphuric acid.

The residue was further dried at $100^\circ C.$ and then weighed.

4.934 grams of the aqueous solution saturated at $14^\circ C.$ left a residue (effloresced K.) which dried at $100^\circ C.$ weighed 0.329 gram.

Therefore 4.605 grams of water at $14^\circ C.$ held in solution 0.329 gram of effloresced kreatinin, corresponding with 0.4338 gram of efflorescent kreatinin ($C_4H_7N_3O.2H_2O$).

Hence the efflorescent kreatinin ($C_4H_7N_3O.2H_2O$) requires 10.6 times its weight of water at $14^\circ C.$ for complete solution.

And the effloresced kreatinin ($C_4H_7N_3O$) requires 14 times its weight of water for complete solution at $14^{\circ}C$.

A very pure specimen of the *tabular kreatinin* α of urine was dissolved to saturation in water kept at $40^{\circ}C$. The saturated solution was then kept at $17^{\circ}C$. for 12 hours. Crystals separated out.

3.9828 grams of the resulting solution saturated at $17^{\circ}C$. was evaporated to dryness *in vacuo* over sulphuric acid. The residue weighed 0.3376 gram. Therefore 3.6452 grams of water at $17^{\circ}C$. held in solution 0.3376 gram of the tabular kreatinin, or 1 gram of tabular kreatinin α of urine is soluble in 10.78 grams of water at $17^{\circ}C$.

Allowing for the different temperatures, it appears, therefore, that the solubility in water of the *efflorescent kreatinin* ($C_4H_7N_3O \cdot 2H_2O$) before efflorescence is practically identical with that of the *tabular kreatinin* α of urine ($C_4H_7N_3O$).

The solubility of kreatinin prepared from the kreatin of flesh is given by Liebig as 1 part in 11.5 parts of water at $16^{\circ}C$.

Solubility of Tabular Kreatinin α of Urine in Alcohol.

A fine specimen of the *tabular kreatinin* α was digested with alcohol of specific gravity 0.795 at its boiling point in a continuous extraction apparatus for some hours. The hot alcoholic solution deposited a number of dendritic crystals on cooling. The solution was left at the ordinary temperature for 20 hours in a well-corked bottle. 10 c.c. of the solution, whose temperature was $17^{\circ}C$., was then drawn off in a pipette and evaporated to dryness over steam. The residue weighed 0.0220 gram. A second 10 c.c. left a residue weighing 0.0216 gram. 7.95 grams of absolute alcohol at $17^{\circ}C$. therefore dissolved 0.022 gram of the tabular kreatinin.

Therefore 1 part by weight of the kreatinin dissolves in 362 parts by weight of absolute alcohol at $17^{\circ}C$.

The solubility of kreatinin prepared from the kreatin of flesh is given by Liebig as 1 part in 102 parts of absolute alcohol at $16^{\circ}C$.

Determination of the Weight of Cupric Oxide reduced by Efflorescent Kreatinin of Urine and by Tabular Kreatinin α of Urine in Boiling Alkaline Solutions.

My object in making these determinations being to ascertain how much of the reducing action of normal urine is to be ascribed to the kreatinin which is present in that secretion, I employed the same method therein as in estimating the cupric oxide reduction effected by the urine itself, viz., Pavy's ammoniacal cupric method.

11.2 c.c. of a solution of the *efflorescent kreatinin of urine*, containing

0.1346 gram of the anhydrous base ($C_4H_7N_3O$) in 60 c.c. were required for the complete reduction of 40 c.c. of Pavy's ammoniacal cupric solution.

Now 40 c.c. of Pavy's solution = 0.02 gram of glucose.

And 11.2 c.c. of the kreatinin solution contain 0.0251 gram of $C_4H_7N_3O$.

Therefore 12.5 grams of the effloresced kreatinin are equivalent to 10 grams of glucose in reducing action upon cupric oxide.

Again 0.5 gram of the *tabular kreatinin* α of urine was dissolved in 250 c.c. of water.

6 c.c. of this solution was required to decolourise 20 c.c. of Pavy's ammoniacal cupric solution (= 0.01 gram of glucose). And 11.9 c.c. was required for 40 c.c. of the cupric solution.

Hence 12 grams of the *tabular kreatinin* α of urine are equivalent to 10 grams of glucose in reducing action upon cupric oxide.

Now
$$\begin{array}{ccc} 2CH_7N_3O : C_6H_{12}O_6 :: 12 : 9.5. \\ = 226 & & = 180 \end{array}$$

From which it appears that 2 mols. of the reducing kreatinin of urine are about equal to 1 mol. of glucose in reducing action upon cupric oxide.

Reduction of Cupric Oxide by Kreatinin from Kreatin of Flesh prepared by Liebig's Method.

62 lbs. of fresh beef, treated by Liebig's process for the extraction of the kreatin, gave some fine crystals of that substance.

3.685 grams of the kreatin obtained in this way was converted into kreatinin hydrochloride by the action of dry hydrogen chloride in Liebig's drying tube.

The resulting hydrochloride was dissolved in 24 parts of water as recommended by Liebig, boiled, and treated with pure lead hydrate added a little at a time. In short, Liebig's directions for preparing kreatinin from the kreatin of flesh were followed as exactly as possible. The kreatinin thus prepared was re-crystallised six times from the smallest possible quantity of cold water by evaporation *in vacuo* over sulphuric acid.

Well-formed anhydrous tabular crystals resulted. In order to compare the cupric oxide reduction of this substance with that of the *natural kreatinin of urine*, a solution was made containing 0.1 gram of the substance in 100 c.c.

27.5 c.c. of this solution decolourised 30 c.c. of Pavy's ammoniacal cupric solution (= 0.015 gram glucose).

Hence 180 parts by weight of glucose are equivalent to 329.94 parts by weight of kreatinin.

Or 1 mol. of glucose is equal to 3 mols. of kreatinin from kreatin of flesh in reducing action upon cupric oxide.

Whereas 1 mol. of glucose is equal to 2 mols. of the natural kreatinin of urine in cupric oxide reducing power.

But the important practical deduction which I draw from the above data is this—that the natural kreatinin of urine is responsible for the bulk of the reducing action exerted by normal human urine, and further that, in my belief, the whole cupric oxide reduction effected by that secretion may be accounted for by uric acid and kreatinin.

Taking 1500 c.c. (= 52·8 fluid ounces) as the average volume of urine passed by a healthy man in the twenty-four hours, and 6 to 7 grams per litre as the average weight of the spherical mercury salt of kreatinin yielded by the normal secretion, then, as the spherical mercury salt contains 20 per cent. of kreatinin ($C_4H_7N_3O$), the weight of anhydrous kreatinin passed in twenty-four hours by a healthy man will vary between 1·8 and 2·1 grams. And as 12 grams of the reducing kreatinin of urine are equivalent to 10 grams of glucose in reducing action upon cupric oxide, it follows that the cupric oxide reduction effected by the urinary kreatinin in twenty-four hours is equivalent to that which would be produced by 1·5 to 1·75 grams of glucose, *i.e.*, by from 23 to 27 grains of glucose in 52·8 fluid ounces of urine.

Accordingly cupric oxide is reduced by the kreatinin of normal urine in the same degree as if the secretion contained from 0·43 to 0·51 grain of glucose in each fluid ounce.

But the total cupric oxide reduction effected by normal human urine is equivalent to from 0·6 to 0·8 grain of glucose per 1 fluid ounce, and of this total reduction one-fourth has been shown by Dr. Pavy to be due to uric acid. Therefore the total reduction is accounted for by the conjoined action of the uric acid and kreatinin.

I can only attribute the little importance which has hitherto been attached to the reducing action of urinary kreatinin, and the low estimates which have been made thereof, to the fact that only one substance having the formula $C_4H_7N_3O$ has hitherto been recognised by physiological chemists, and that therefore the properties of the kreatinin obtained by Liebig's process from kreatin of flesh have been supposed to be identical with those of the *natural kreatinin of urine*.

I shall prove in Part II of this paper that the properties of the kreatinins obtained from the kreatin of urinary kreatinin itself, by treating it according to Liebig's direction, are in many respects different from those of the natural base. It will be sufficient at present to emphasise some of the differences in properties which I have observed between the natural kreatinin of urine and the kreatinin described by Liebig.

1st. As regards *reducing action*.

I have prepared kreatinin from flesh kreatin by Liebig's process,

and have found differences in the reducing action given by the products. But the specimen which agreed best with the description given by Liebig was destitute of reducing action.

Professor W. N. Hartley has compared the absorption spectrum of this specimen with that of a specimen of *tabular kreatinin* α of urine for me, and his results are published in an Appendix to this paper.

2nd. The *platinum salts* differ.

In all the descriptions of "kreatinin platinic chloride" that I have found, the salt is described as *anhydrous*. I have subjected the platinum salt of urinary kreatinin to complete analysis, and find that it contains 2 mols. of water of crystallisation.

3rd. The *solubilities in water and alcohol* differ.

The solubility in alcohol of kreatinin (Liebig) is 1 part in 102 parts of alcohol at 16° C.

The solubility in alcohol of *tabular kreatinin* α of urine is 1 part in 362 parts of alcohol at 17° C.

Kreatinin (Liebig) dissolves in 11.5 parts of water at 16° C.

Tabular kreatinin α of urine dissolves in 10.78 parts of water at 17° C.

It appears, therefore, that the tabular kreatinin of urine is slightly more soluble in water, and vastly less soluble in alcohol than the artificial base described by Liebig.

I will conclude this part of my work by laying stress upon the importance of the precipitation of kreatinin from urine as spherical mercury salt, not only as a convenient method of obtaining the natural base in a state of purity, but also as a means of estimating with accuracy the *quantity* of kreatinin in a given volume of the secretion; and at the same time the amount of reduction exerted by the natural base. In Part II will be found an account of the bases obtainable from the kreatin of urinary kreatinin.

PART II.

On the Kreatinins derived from the Dehydration of Urinary Kreatin.

When the kreatinin of urine is boiled in dilute aqueous solution in proportion 1 : 1000 by weight of water, it is gradually converted into kreatin, which may be crystallised out by concentrating at the boiling temperature. If the first crop of kreatin be separated from the mother-liquor, the latter, diluted largely and again concentrated, yields a further crop of crystals, and this may be repeated as long as any kreatin crystallises out. The whole of the crystals thus obtained are then dissolved together in boiling water, the solution kept boiling with animal charcoal till colourless, filtered, and concentrated. On cooling the pure urinary kreatin crystallises out.

Determinations of Water of Crystallisation in Urinary Kreatin.

Weight of air-dried crystals in grams.	Weight lost at 100° C.	Weight lost by 100 parts.	H ₂ O in 100 parts of C ₄ H ₉ N ₃ O ₂ .H ₂ O.
3·547	0·4415	12·45	12·08
2·955	0·363	12·28	12·08

These results agree with the formula C₄H₉N₃O₂.H₂O.

Conversion of Urinary Kreatin into Kreatinin Hydrochloride by Liebig's Process.

Weighed portions of the air-dried urinary kreatin were introduced into a counterpoised Liebig's drying tube, connected at one extremity with apparatus for the delivery of dry air or dry hydrogen chloride at will, and at the other extremity with a counterpoised bulb apparatus containing sulphuric acid. In some experiments the kreatin was first dried at 100° C. in dry air, and then subjected to the action of dry HCl at 100° C.; in others the crystals were at once acted on by HCl, without previous removal of their water of crystallisation. The final products were found to have similar properties in all the experiments. Dry air was, of course, kept passing in all cases at 100° C., until no more water was expelled, *i.e.*, until the weight of the sulphuric acid bulbs in front of the kreatin tube became constant.

The following are some of the quantitative results :—

Weight of kreatin taken in grams.	Weight of H ₂ O collected.	Weight of kreatinin hydrochloride found.	Theoretical weight of H ₂ O expelled.	Theoretical weight of kreatinin hydrochloride.
(Cryst.) 1·066	0·276	1·076	0·259	1·0696
(Anhydrous) 3·1055	0·412	3·507	0·426	3·544
(Anhydrous) 2·592	0·364	2·950	0·356	2·957
(Cryst.) 2·659	0·594	2·708	0·642	2·661
(Cryst.) 5·063	1·220	5·078	1·223	5·0799

The above results sufficiently prove that when urinary kreatin is acted upon by dry HCl at 100° C., the change is expressed by the equation C₄H₉N₃O₂.H₂O + HCl = C₄H₇N₃O.HCl + 2H₂O.

Comparison between Kreatinin Hydrochloride prepared by Liebig's Process from Urinary Kreatin and the Kreatinin Hydrochloride obtained from the Spherical Mercury Salt of Urine.

When the contents of the tube, in which urinary kreatin has been converted into kreatinin hydrochloride by dry HCl at 100°C. , are dissolved in cold water, three times its weight of which is sufficient for complete solution, there are obtained on evaporation *in vacuo* over sulphuric acid transparent flattened prisms, which rapidly become opaque on exposure to common air by efflorescence.

1.26 gram of these crystals (air-dried and weighed at once) lost 7.06 per cent. after 48 hours' exposure to common air at 15°C. Being finally dried at 100°C. , they were found to have lost altogether 10.9 per cent.

The formula $\text{C}_4\text{H}_7\text{N}_3\text{O.HCl.H}_2\text{O}$ requires 11.04 per cent. H_2O . In this respect, kreatinin hydrochloride obtained by Liebig's process from urinary kreatin, resembles the kreatinin hydrochloride obtained from kreatinin of flesh by the same process.

Thus 0.323 gram of air-dried transparent square plates of kreatin hydrochloride, prepared by me from beef by Liebig's process, and crystallised by spontaneous evaporation from cold aqueous solution, lost 12.4 per cent. of their weight when dried at 100°C. , which is rather more than is required by the formula $\text{C}_4\text{H}_7\text{N}_3\text{O.HCl.H}_2\text{O}$. The occurrence of water of crystallisation in the crystals obtained by spontaneous evaporation of cold aqueous solutions of kreatinin hydrochloride obtained artificially by Liebig's process from kreatin (whether urinary or sarcous in its origin) is an important means of distinguishing these artificial hydrochlorides from the natural hydrochloride of urinary kreatinin, the crystals of which I have invariably found to be anhydrous, even when the utmost care is taken to avoid heating their solution (*vide* Part I of this paper).

Description of the Preparation of Kreatinins from the Kreatinin Hydrochloride produced from Urinary Kreatin.

The same process was adopted in liberating the kreatinin from the artificial hydrochloride as in the case of the hydrochloride of the kreatinin of urine itself. The concentrated aqueous solution was well stirred with excess of pure lead hydrate, at the ordinary temperature, till the reaction to litmus became strongly alkaline. The liquid was then filtered and evaporated *in vacuo* over sulphuric acid. In some cases this treatment produced efflorescent kreatinin, exactly resembling in crystalline form and in composition the efflorescent kreatinin of urine ($\text{C}_4\text{H}_7\text{N}_3\text{O.2H}_2\text{O}$); whilst in other cases anhydrous tabular crystals were formed, which were found to be crystallographically identical with the tabular natural kreatinin of urine. I

have been unable to ascertain the cause of the formation of these two bodies in different experiments, but I shall show presently that they are very easily convertible the one into the other.

For the crystallographic examination of the kreatinin crystals prepared by me from urine and from the kreatin of urine, I am indebted to the kindness and patience of Mr. L. Fletcher of the Mineralogical Department of the British Museum. He has found that the tabular crystals of kreatinin, both natural and artificial, are identical in their angles with those measured by Kopp and Heintz. He has also made for me (with the aid of Mr. H. A. Miers) what, I suppose, are the first measurements of the efflorescent kreatinin. Mr. Fletcher's report is as follows :—

Kreatinin (measured by Fletcher).

Anhydrous tabular K. from urine and from urinary kreatin by Liebig's process. Thin, rectangular, nearly square tables.

System—Monosymmetric.

Elements— $a : b : c :: 1.235 : 1.116 ; \gamma = 69^\circ 47'$.

Forms observed— $a\{100\}$, $c\{001\}$, $m\{110\}$, $d_1\{\bar{1}01\}$.

The development of the forms is illustrated by Figs. 2 and 3.

The form d was only observed in one of the crystals.

There is an easy cleavage parallel to $a_1\{100\}$.

FIG. 2.

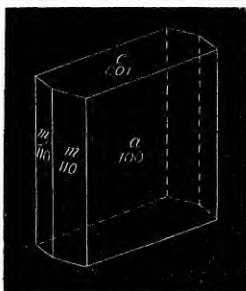
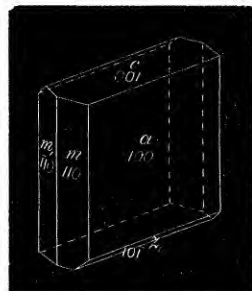


FIG. 3.



Observed.			Kopp.	Heintz.
Angles.	Mean.	Limiting values.		
$ac \dots$	$69^\circ 47'$	$69^\circ 35' - 70^\circ 3'$	$69^\circ 24'$	$69^\circ 57' - 70^\circ 30'$
$cd_1 \dots$	$50^\circ 58'$	$50^\circ 50' - 51^\circ 6'$		
$am \dots$	$49^\circ 13'$	$48^\circ 48' - 49^\circ 34'$		
$mm_1 \dots$	$81^\circ 34'$	$81^\circ 12' - 81^\circ 53'$	$81^\circ 40'$	$81^\circ 40'$, sometimes $37'$ or $38'$.

The crystals were thus identical in their angles with those measured by Kopp and Heintz.

Efflorescent Kreatinin, ($C_4H_7N_3O \cdot 2H_2O$). *Measured by L. Fletcher and H. A. Miers.*

Long prisms.

System—monosymmetric.

Elements— $a : b : c :: 0.6114 : 1 : ?$; $\gamma = 80^\circ 18'$.

Forms observed— $b\{010\}$, $c\{001\}$, $m\{110\}$, $h\{120\}$, $f\{130\}$.

Angles found.	Observed.	Calculated.
bm	$58^\circ 55\frac{1}{2}'$	$58^\circ 55\frac{1}{2}'$
cm	$81^\circ 42'$	$81^\circ 42'$
bh	$39^\circ 40\frac{1}{2}'$	$39^\circ 41'$
bf	$28^\circ 7'$	$28^\circ 57'$
ch	$83^\circ 45'$	$83^\circ 49'$
cf	$85^\circ 13'$	$85^\circ 19'$

Analyses of Kreatinins from Urinary Kreatin by Liebig's Process.

Determinations of Water of Crystallisation in the Efflorescent Kreatinin from Urinary Kreatin.

The same difficulty was experienced in making these determinations as in the case of the efflorescent kreatinin obtained directly from urine, on account of the extreme rapidity of the efflorescence.

Weight of efflorescent kreatinin taken (in grams).	Loss of weight at $100^\circ C$.	Loss of weight in 100 parts.	Theoretical loss for $C_4H_7N_3O \cdot 2H_2O$.
0.726	0.1736	23.91	24.16
0.465	0.114	24.51	24.16

The formula $C_4H_7N_3O \cdot 2H_2O$ receives additional support from the results of the ultimate analysis of the efflorescent kreatinin, before it has undergone efflorescence.

Combustion of Efflorescent Kreatinin ($C_4H_7N_3O \cdot H_2O$) from Urinary Kreatin.

Weight of substance taken in grams.	Hydrogen found.	Carbon found.	Hydrogen in 100 parts found.	Carbon in 100 parts found.
0.284	0.0203	0.0917727	7.16	32.31

	Found in	Required for
	100 parts.	$C_4H_7N_3O \cdot 2H_2O$ ($C_4H_{11}N_3O_3$).
Carbon	32·31	32·21
Hydrogen	7·16	7·30

The ultimate analysis of the efflorescent kreatinin after efflorescence proves that the composition of the effloresced substance is identical with that of the anhydrous tabular kreatinin from urinary kreatin.

Ultimate Analysis of Effloresced Kreatinin from Kreatin of Urine and of Tabular Kreatinin from the same source.

Description of substance.	Weight of substance in grams.	Weight of hydrogen found.	Weight of carbon found.	H in 100 parts.	C in 100 parts.
Effloresced kreatinin from kreatin of urine	0·215	0·0137	0·09136	6·404	42·49
Tabular kreatinin from kreatin of urine	0·312	0·0195	0·1342	6·26	43·01
Tabular kreatinin from kreatin of urine	0·1515	0·0100	0·0641	6·60	42·47

Description of substance.	Weight of substance in grams.	Vol. of nitrogen at 0° C. and 760 mm.	Weight of nitrogen in grams.	Nitrogen in 100 parts.
Effloresced kreatinin from urinary kreatin	0·154	c.c. 45·574	0·057171	37·123
Tabular kreatinin from urinary kreatin	0·1505	44·53	0·055862	37·117

These results agree well with the formula $C_4H_7N_3O$, as shown by the following table:—

	Found in tabular kreatinin from urinary kreatin.	In effloresced kreatinin from same source.	Required for $C_4H_7N_3O$ in 100 parts.
	Mean.		
Carbon	42·31 } 42·66	42·49	42·478
	43·01 }		
Hydrogen	6·60 } 6·43	6·404	6·194
	6·26 }		
Nitrogen	.. 37·117	37·123	37·168
Oxygen (by difference) ..	.. 13·793	(differ.) 13·983	14·160
	.. 100·000	100·000	100·000

Platinum and Gold Salts of Kreatinins from Urinary Kreatin.

When a solution of tabular kreatinin, prepared from urinary kreatin by Liebig's process, and which has re-crystallised in the tabular form from cold aqueous solution, is acidulated with hydrochloric acid, mixed with aqueous solution of platinic chloride and evaporated *in vacuo* over sulphuric acid, a crystalline platinum salt is formed. The crystals are paler in colour than the platinum salt of the tabular kreatinin of urine, and require nearly twice as much water to dissolve them. The salt is easily purified by washing away the excess of platinic chloride from it with strong alcohol, and then re-crystallising from watery solution.

Like the platinum salt of the kreatinin of urine, it contains two molecules of water of crystallisation.

0.162 gram of the salt became opaque at 100° C., losing 0.009 gram of water.

This corresponds with 5.55 per cent.

The formula $2\{C_4H_7N_3O.HCl\}PtCl_4.2H_2O$ requires 5.34 per cent.

Comparison between the Platinum Salts of Tabular Kreatinin α of Urine and Tabular Kreatinin α from the Urinary Kreatin, as regards solubility in Water.

Weight of solution (in grams) saturated at 15° C.	Name of salt taken.	Weight of dried at 100° C.	Parts by weight of water which dissolve 1 part of salt at 10° C.
9.837	Platinum salt of tabular $K\alpha$ of urine	0.652	14.1
3.641	Platinum salt of tabular $K\alpha$ from urinary kreatin	0.143	24.4

Thus the platinum salt of the natural kreatinin is nearly twice as soluble in water as that of the artificial base.

Neither the efflorescent kreatinin of urine, nor the efflorescent kreatinin from urinary kreatin form platinum salts of any definite nature. After treatment with alcohol the crystalline matter which remains upon spontaneous evaporation of mixed chlorides is found to be a mixture of a little yellow granular matter with transparent colourless needles, probably the hydrochloride of the kreatinin.

With auric chloride, however, the efflorescent kreatin, and also the tabular anhydrous kreatinin from urinary kreatin, give fine gold salts, having the formula $C_4H_7N_3O.HCl_4.AuCl_3$, and crystallising in thin yellow lustrous plates.

The gold salt of the efflorescent kreatinin from kreatin is darker in

colour than that of the efflorescent kreatinin of urine, but it has the same composition, as shown from the following analysis:—

Gold Salt of Efflorescent Kreatinin from Kreatin.—0.520 gram gave 0.226 gram Au, equivalent to 43.461 per cent.

	Found.	Required for $C_4H_7N_3O \cdot HCl \cdot AuCl_3$.
Au	43.461	43.43

The gold salts of natural kreatinins from urine may be readily distinguished from those of artificial kreatinins obtained from the urinary kreatin, by the action of ether, which decomposes the latter, but has no action upon the former. When applied to the crystals of artificial kreatinin auric chloride, ether renders them opaque, dissolving out auric chloride and leaving the hydrochloride of the kreatinin.

The crystals of gold salt of the natural kreatinin on the contrary remain unchanged under ether.

Also, if ether be added to the alcoholic solution of the natural kreatinin in auric chloride, no change takes place, but it causes a precipitate of the kreatinin hydrochloride when added to the alcoholic solutions of the gold salts of the artificial kreatinins.

Solubility in Water and Alcohol of Tabular Kreatinin α from Urinary Kreatin.

Weight of solution in water, saturated at 17° C.	Weight of residue dried at 100° C.	Parts by weight of water at 16.5° C. required to dissolve 1 part of base.
5.0412 grams	0.4314 gram	10.68 parts

This agrees very closely with the solubility of natural tabular kreatinin in water, which requires 10.78 parts at 17° C.

The solubility of the artificial tabular kreatinin in alcohol is, however, greater than that of the natural base. At 18.5° C., 324 parts of absolute alcohol dissolve 1 part by weight of tabular kreatinin α from urinary kreatin, whereas the natural tabular kreatinin of urine requires 362 times its weight at 17° C.

Effects of Re-crystallisation from Aqueous Solution at Different Temperatures of Kreatinins obtained from Urinary Kreatin.

There appear to be four varieties of kreatinin obtainable from urinary kreatin.

(1.) Efflorescent kreatinin α , which, having effloresced, recrystallises in the same form after spontaneous evaporation of its cold aqueous solution.

(2.) Efflorescent kreatinin β , which, having effloresced, recrystallises in the tabular anhydrous form from cold aqueous solution.

(3.) Tabular kreatinin α , which recrystallises in the same form when its cold aqueous solution is evaporated *in vacuo* over sulphuric acid.

(4.) Tabular kreatinin β , which recrystallises in the efflorescent form when its cold aqueous solution is spontaneously evaporated.

There is no apparent difference between the crystals of (1) and (2), nor any between those of (3) and (4). Yet, when the conditions of solution and evaporation are kept as similar as possible, there is the remarkable difference described in the products of re-crystallisation. These four substances may be obtained as follows:—

(1.) Efflorescent kreatinin α is the product usually obtained after the treatment of the kreatinin hydrochloride, prepared from urinary kreatin, by Liebig's process, with pure lead hydrate at the ordinary temperature. After it has effloresced, we may obtain from it either (3) or (4).

The tabular kreatinin α is formed if the solution of the effloresced crystals is made at 100° C., even though the subsequent evaporation be conducted at the ordinary temperature.

The tabular kreatinin β is the product if the effloresced crystals are dissolved in water at 60° C.

The weight of both these forms of crystals is always identical with that of the effloresced kreatinin from which they were obtained.

(2.) Efflorescent kreatinin β is obtained by dissolving tabular kreatinin α in water at 60° C., and then subjecting the solution to evaporation *in vacuo* over sulphuric acid. Efflorescent kreatinin is never obtained by deposition on cooling from a hot solution.

From tabular kreatinin α tabular kreatinin β is obtained by dissolving in boiling water, and then evaporating the solution at the ordinary temperature.

The following are some results of re-crystallisation of artificial urinary kreatinin:—

Nature of crystals.	Dissolved in	Evaporated.	Product.
Tabular K.	Water at 60° C.	In vacuo ...	Efflorescent K β .
Efflorescent K β ..	Cold water. . . .	Do. ...	Tabular K β .
Tabular K β	Do.	Do. ...	Efflorescent K α .

In this case alternate crystallisation of tabular and efflorescent kreatinin took place twice, the kreatinin becoming finally permanently

efflorescent. If repeated re-crystallisations by spontaneous evaporation from cold aqueous solution be made, the kreatinin tends ultimately to assume the efflorescent form.

By redissolving at 60° C., we may, at any time, cause the production of temporarily tabular kreatinin β , which swings back to the permanently efflorescent state after one or two re-crystallisations from cold aqueous solution; or by redissolving in water at 100° C., we may produce tabular kreatinin α , which re-crystallises in the same form indefinitely from cold aqueous solution.

It is extremely remarkable, however, that the efflorescent kreatinin is obtained by dissolving tabular kreatinin α in water at 60° C., and then evaporating spontaneously.

Condition of the Two Molecules of Water in the Efflorescent Kreatinin.

Although this water behaves like the water of crystallisation in an efflorescent salt in many respects, and although it does not appear in the gold salt formed by the efflorescent kreatinin, yet there are reasons for supposing that either the kreatinin in the efflorescent kreatinin differs from the tabular anhydrous kreatinin; or else that the water is more closely combined with the kreatinin than ordinary water of crystallisation. For instance—

1st. The solubility in water of the efflorescent kreatinin *before efflorescence* is the same as that of the anhydrous tabular kreatinin.

2nd. When re-crystallised by evaporation of its *cold solution in vacuo* after being dissolved at the boiling temperature, anhydrous tabular kreatinin is produced.

3rd. There is difficulty in forming a platinum salt of efflorescent kreatinin, none in forming the platinum salt of the tabular kreatinin.

4th. When re-crystallised from boiling alcoholic solution, the efflorescent kreatinin forms needle-shaped crystals exactly resembling the original base, and entirely different from those which are produced in the alcoholic solution of the tabular kreatinin.

Cupric Oxide Reduction effected by the Tabular Kreatinin from Urinary Kreatin.

10.8 c.c. of a solution containing 0.1785 gram of tabular kreatinin α from kreatin of urine in 60 c.c., were required to decolourise 40 c.c. of Pavy's ammoniacal cupric solution (= 0.02 gram of glucose), *i.e.*—

10 parts by weight of glucose reduce as much CuO as 16 parts by weight of kreatinin (tabular α), or

2 mols. of glucose are equivalent to 5 mols. of kreatinin in reducing action upon CuO.

A specimen of *tabular kreatinin* β from urinary kreatinin was found to have exactly the same reducing action as the above.

Tabular Synopsis of Different Kreatinins.

	Efflorescent K. of urine. $C_4H_7N_3O_2 \cdot 2H_2O$.	Tabular Kreatinin of urine. $C_4H_7N_3O$.	Efflorescent K. from urinary kreatin. $C_4H_7N_3O_2 \cdot 2H_2O$.	Tabular K. from urinary kreatin. $C_4H_7N_3O$.	"Kreatinin." —— (Liebig).
Solubility in water...	1 in 10.6 at 14° C.	1 in 10.78 at 17° C.		1 in 10.68 at 16.5° C.	1 in 11.5 at 16° C.
Solubility in alcohol.		1 in 362 at 17° C.		1 in 324 at 18.5° C.	1 in 102 at 16° C.
Platinum salt.....	Indefinite or decom- posed by alcohol	$2(C_4H_7N_3O \cdot HCl)$ $PtCl_4 \cdot 2H_2O$	Indefinite, or decom- posed by alcohol	$2(C_4H_7N_3O \cdot HCl)$ $PtCl_4 \cdot 2H_2O$	$2(C_4H_7N_3O \cdot HCl)$ $PtCl_4$
Solubility of platinum salt in water		1 in 14.1 at 15° C.		1 in 24.4 at 15° C.	
Gold salt.....	Unchanged by ether	Unchanged by ether	Decomposed by ether	Decomposed by ether	
Reduction of CuO compared with that of glucose	4 molecules $C_4H_7N_3O$ = 2 mols. glucose	4 molecules $C_4H_7N_3O$ = 2 mols. glucose.	5 molecules $C_4H_7N_3O$ = 2 mols. glucose	5 molecules $C_4H_7N_3O$ = 2 mols. glucose.	

Reducing Action of a Specimen of Kreatinin from Kreatin of Beef.

The kreatin from which this kreatinin was prepared was extracted by myself from beef (62 lbs.) by Liebig's process, and I followed Liebig's directions as exactly as possible in converting it into kreatinin. The product, however, required 390.2 times its weight of alcohol at 15° C. for solution, and its platinum salt contained water of crystallisation, so that the base does not agree in properties with that described by Liebig.

The reducing action of this base was feebler than that of the artificial kreatinin from the kreatin of urine. It required 3 mols. of this kreatinin to reduce as much cupric oxide as 1 mol. of glucose.

Thus, comparing the reducing action of kreatinins, we have—

Tabular and efflorescent	}	Kreatinins from urine.	4	molecules	=	2	mols.	glucose.
Tabular α and β	}	Kreatinins from urine and any kreatin.....	5	"	"	"	"	"
Kreatinin from kreatin of flesh.....	6	"	"	"	"	"	"	"

It is evident, therefore, that the reducing action of the natural kreatinin is much greater than any of the artificial kreatinins which I have examined.

In conclusion, I think it is proved—

(1st.) That the most active reducing agent in normal urine is the urinary kreatinin.

(2nd.) That the properties of kreatinins artificially prepared cannot be considered as identical with those of the natural base; and

(3rd.) That there is strong presumptive evidence against the existence of sugar in normal human urine.

In this paper I have confined my attention to the differentiation of kreatinins by the study of their physical properties, reactions, &c. I hope shortly to study the substitution products of the various substances I have described, with a view to the construction of rational formulæ, &c.

Appendix by Professor W. N. HARTLEY, F.R.S.

On the Absorption-spectrum of a Base from Urine.

The base which Mr. G. S. Johnson has separated from urine is regarded as isomeric with kreatinin artificially prepared by Liebig's process; it has therefore been considered of interest to ascertain the character of its absorption-spectrum and more especially to compare the specific absorptive power of the two substances. The method of examination was that described in the 'Proceedings of the Royal

Society,' vol. 33, p. 1, with the modifications described in the 'Journal of the Chemical Society,' vol. 47, pp. 685—757. The absorption-curves have been drawn in the same way as those figured in the latter publication.

The two bodies, kreatinin and the *base* from urine, are compounds with a very similar constitution, as is apparent from the two curves accompanying this note. The absorption-bands are not caused by the condensation of the carbon atoms as in aromatic derivatives of benzene, but by the condensation of the numerous oxygen and nitrogen atoms, as is the case with uric acid. The absorption-bands, though definite, have no great persistency, but the intensity of the total actinic absorption is great, the band of kreatinin becoming visible when 2 mm. of a solution were examined containing little more than 1 part of the substance in 1000 of water. With the *base* from urine 2 mm. of solution show a band when the same quantity is contained in 5000 of water.

The Absorption Spectra of Kreatinin.* 0.0113 gram dissolved in 10 c.c. of water, or a milligram-molecule in 100 c.c.

Thickness of layer of liquid	20 mm.		15 mm. and 10 mm.		5 mm. and 4 mm.	
	Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.
Description of spectrum..... {						
Continuous, strong, to	3080	3245	3080	3245	3080	3245
" fairly strong, to	3832	2609	3832	2608	3890	2568
Line visible at	..	..	3890	2568	..	..
Rays extend to	..	..	..	..	3936	2538
Thickness of layer of liquid	3 mm.		2 mm.		1 mm.	
Continuous, strong, to	3080	3245	3080	3245	3080	3245
" fairly strong, to	3890	2568	3890	2568	3890	2568
Rays extend to	4055	2466	4141	2415	4141	2415
ABSORPTION BAND	..	..	4141 to 4331	2415 to 2310	Absorption weaker.	..
Spectrum ends at	..	..	..	..	4426	2259

* This specimen of kreatinin was prepared by the action of dry HCl at 100° C. upon kreatin (Liebig's process). It gave all the reactions described by Liebig, but no reduction of picric acid or mercuric oxide at the ordinary temperature. With Nessler's reagent, it gave a yellow precipitate, becoming red on standing.—G. S. J.

Kreatinin—*continued*. 0.0113 gram dissolved in 50 c.c. of water, or a milligram-molecule in 500 c.c.

	5 mm.	4 mm. and 3 mm.		2 mm. and 1 mm.	
		Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.
Thickness of layer of liquid					
Description of spectrum	The same spectrum as with 1 mm. of the more dilute solution.				
Strong and fairly strong, to Rays extend to		3890 4566	2568 2190	3890 4660	2568 2146

Absorption Spectrum of the Base from Urine. 0.0113 gram. dissolved in 10 c.c. of water, or a milligram-molecule in 100 c.c.

	20 mm., 15 mm., and 10 mm.		5 mm. and 4 mm.		3 mm., 2 mm., and 1 mm.	
	Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.
Thickness of layer of liquid.....						
Description of spectrum..... {						
Continuous, strong and fairly strong, to Spectrum extends	3647 3768	2740 2653	3768 3882	2653 2609	3882 3890	2609 2568

Base from Urine—*continued*. 0.0113 gram dissolved in 50 c.c. of water, or a milligram-molecule in 500 c.c.
5 mm. of this yield the same spectrum as 1 mm. of the more dilute solution.

	4 mm. and 3 mm.		2 mm.		1 mm.	
	Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.	Oscillation-frequencies.	Wave-lengths.
Thickness of layer of liquid.....						
Description of spectrum..... {						
Continuous, strong and fairly strong, to Spectrum extends to.....	3890 3936	2568 2538	3890 ..	2568 ..	..	..
Weak spectrum to.....	..	..	4055	2466	..	..
ABSORPTION BAND from.....	..	..	4055 to 4331	2466 to 2310	..	..
Spectrum extends to.....	..	..	4660	2146	4660	2146

FIG. 4.

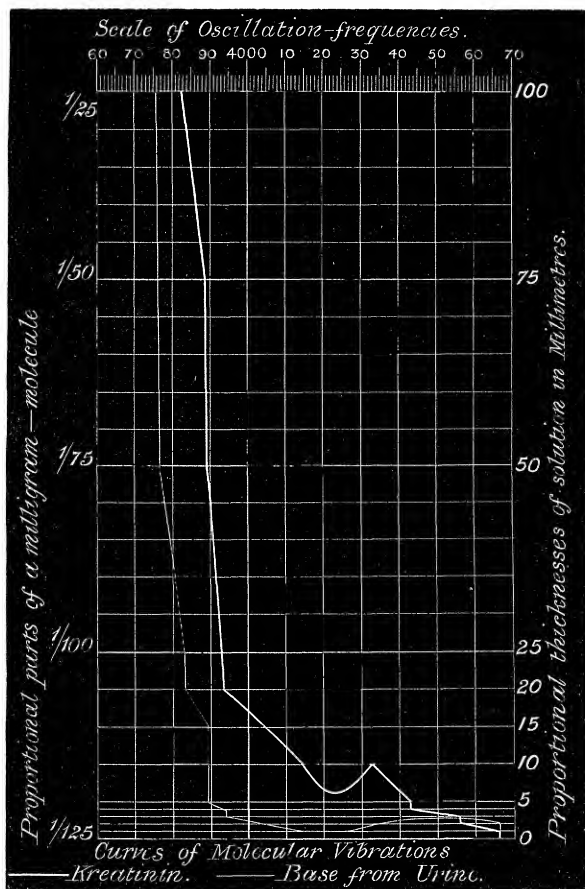
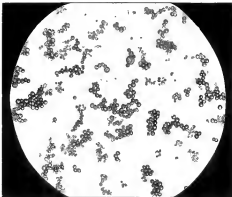


FIG. 1.



Microphotograph of Spherical Mercury Salt of Creatinine, precipitated from fresh normal human urine. $\times 1400$ diameters.

FIG. 2.



Fig. 3.



FIG. 4.

Scale of Oscillation-frequencies.

10 20 30 40 50 60 70 80 90 100

